

Considering how biological sex impacts immune responses and COVID-19 outcomes

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Abstract | A male bias in mortality has emerged in the COVID-19 pandemic, which is consistent with the pathogenesis of other viral infections. Biological sex differences may manifest themselves in susceptibility to infection, early pathogenesis, innate viral control, adaptive immune responses or the balance of inflammation and tissue repair in the resolution of infection. We discuss available sex-disaggregated epidemiological data from the COVID-19 pandemic, introduce sex-differential features of immunity and highlight potential sex differences underlying COVID-19 severity. We propose that sex differences in immunopathogenesis will inform mechanisms of COVID-19, identify points for therapeutic intervention and improve vaccine design and increase vaccine efficacy.

The COVID-19 pandemic, caused by the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in millions of infections and hundreds of thousands of deaths worldwide. Human biological sex plays a fundamental role in heterogeneous COVID-19 outcomes. Sex, defined as male, female or intersex on the basis of sex chromosome complement, reproductive tissues (ovaries or testes) and sex steroid hormone (oestrogen, progesterone and testosterone) concentrations, is a multidimensional biological characteristic that shapes infectious disease pathogenesis. We discuss how sex differences in basic molecular and cellular mechanisms can be leveraged to define the immune response to infection with SARS-CoV-2.

Sex differences in COVID-19 severity

The precise drivers of death, regardless of sex, in COVID-19 remain unknown. There appears to be a subset of patients in whom high levels of dysregulated inflammation lead to severe multisystem organ pathology^{1,2}. A postviral inflammatory syndrome has also emerged in children with COVID-19 (REFS^{3,4}). As a result, research on therapeutics has focused on both antiviral and immunomodulatory pathways^{2,5}

with the goal of achieving an optimized balance in immune response induction and resolution. Unfortunately, most studies fail to consider the sex of the patients, which may mask therapeutic targets.

Evidence of sex differences in COVID-19 severity emerged in China, where hospital admissions and mortality were higher among males than females^{6–8}. In South Korea, where community testing was widespread, females represented ~60% of those testing positive for SARS-CoV-2, suggesting that females acquire infection, despite having a lower case fatality rate (CFR)^{9,10}. In the United States, where testing was prioritized for people with symptomatic disease, the diagnosis rates were similar in males and females, but males had 1.5 times higher mortality¹¹.

A male bias in COVID-19 mortality is currently reported in 37 of the 38 countries that have provided sex-disaggregated data (FIG. 1a). Our analyses show that the average male CFR across 38 countries is 1.7 times higher than the average female CFR ($P < 0.0001$) (male CFR 7.3 (95% CI 5.4–9.2); female CFR 4.4 (95% CI 3.4–5.5)), which is consistent with other reports^{12,13}. There is increased risk of death for both sexes with advancing age, but at all ages above 30 years males have a significantly higher risk of

death than females ($P < 0.05$) (FIG. 1b). A male predominance of deaths from COVID-19 is consistent with what was observed in the prior SARS^{14,15} and Middle East respiratory syndrome (MERS)¹⁶ epidemics (caused by SARS-CoV and MERS-CoV, respectively). Although gender-related social factors, including smoking, health care-seeking behaviours and some co-morbid conditions, may impact the outcomes of COVID-19 (REFS^{6,17}) and contribute to male–female differences in disease severity, the cross-cultural emergence of increased risk of death for males points to biological risk determinants. In animal models of SARS-CoV infection, differences in mortality between male and female mice were observed and were attributed to steroid hormones¹⁸. Multiple dimensions of biological sex, including sex steroids, sex chromosomes and genomic and epigenetic differences between males and females, impact immune responses^{19–26} and may affect responses to SARS-CoV-2 infection²⁷.

Ageing, sex and COVID-19

Although advancing age is associated with greater risk of death in both sexes, the male bias remains evident (FIG. 1b). An analysis of COVID-19 data from Italy, Spain, Germany, Switzerland, Belgium and Norway reveals that among all age groups older than 20 years, fatality rates are greater for males than females²⁸. By contrast, male–female differences in the rate of confirmed SARS-CoV-2 infections are age dependent in all countries, being greater among females aged between 10 and 50 years and greater among males before the age of 10 years and after the age of 50 years²⁸. The age-related male–female differences in confirmed cases of SARS-CoV-2 infections are consistent with reported confirmed cases of seasonal and pandemic influenza A virus infections in Australia and Japan^{29,30}. We interpret these data to suggest that biological sex differences contribute to male-biased death, but gender-associated risk of exposure may affect rates of infection differently for males and females.

With a focus on biology, the impact of age on susceptibility to severe COVID-19 needs to be parsed, with both immunosenescence and dysregulation of innate immune responses as potential

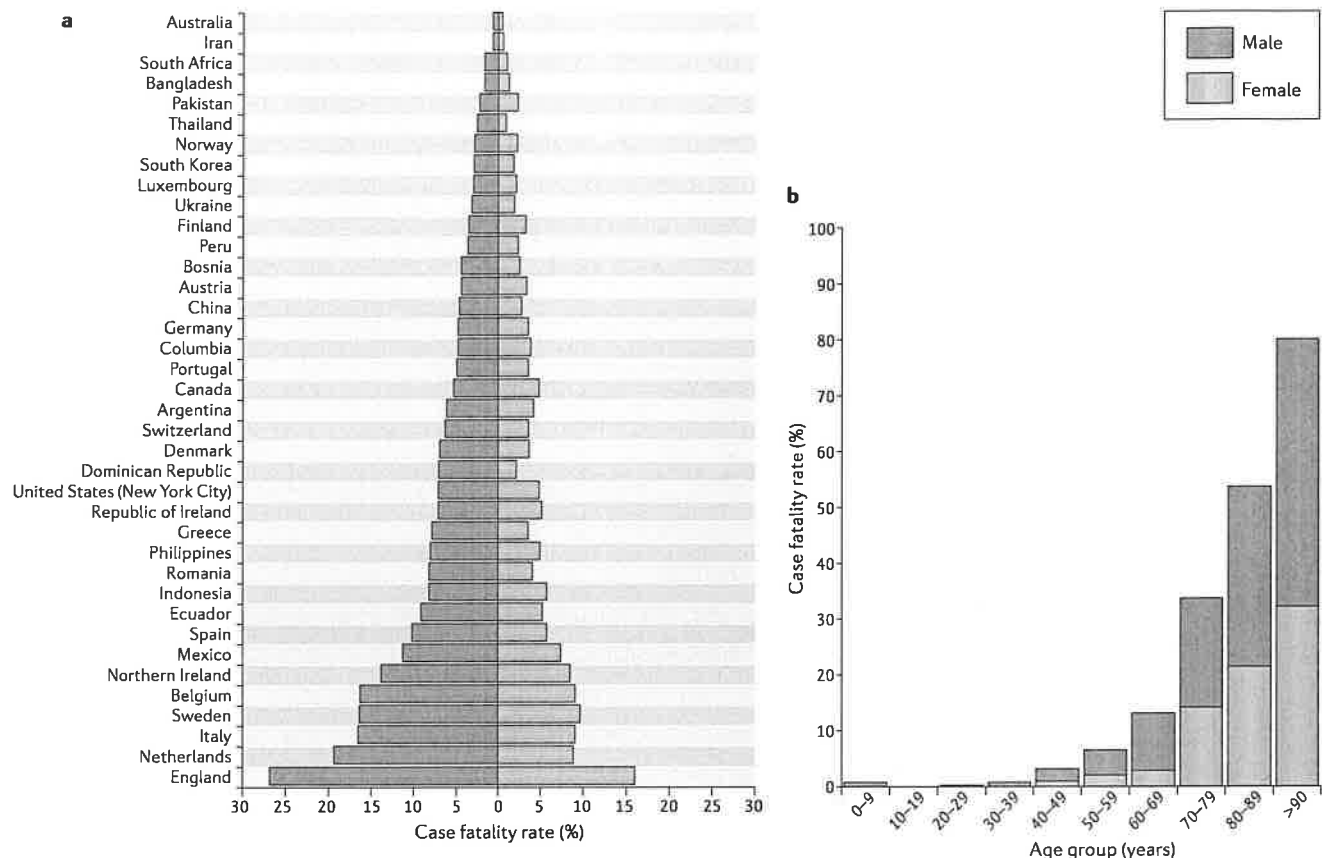


Fig. 1 | Comparative analyses of COVID-19 case fatality rates by country, sex and age. a | COVID-19 case fatality rates (CFRs) for males and females across 38 countries or regions reporting sex-disaggregated data on COVID-19 cases and deaths. CFR was calculated as the total number of deaths divided by the total number of cases for each sex multiplied by 100. The male CFR is higher than the female CFR in 37 of the 38 regions, with an average male CFR 1.7 times greater than the average female CFR ($P < 0.0001$, Wilcoxon signed rank test). **b** | Average COVID-19 CFRs for males and females stratified by age. The data represent 12 countries currently reporting sex- and age-disaggregated data on COVID-19 cases and deaths (Australia, Colombia, Denmark, Italy, Mexico, Norway, Pakistan, Philippines, Portugal, Spain, Switzerland and England). The COVID-19 CFR increases for both sexes with advancing age, but males have a significantly higher CFR than females at all ages from 30 years ($P < 0.05$, Wilcoxon signed rank test). The data were obtained from Global Health 50/50 and official government websites of each respective country on 7 May and 8 May 2020. For more information on the data source for a specific country, please contact the corresponding author.

mechanisms^{31,32}. Biological sex differentially affects ageing of the immune system³³, in part through changing concentrations of sex steroids³⁴. In addition to reduced concentrations of sex steroids, an age-related mosaic loss of chromosome Y in leukocytes can alter transcriptional regulation of immunoregulatory genes³⁵. Whether sex differences in the genomic signatures of aged immune cells translate to functional differences in the response to SARS-CoV-2 infection requires attention.

Sex differences in immune responses

Biological sex affects innate and adaptive immune responses to self and foreign antigens, resulting in sex differences in autoimmunity as well as in responses to infections and vaccines^{36,37}. Immune cell subsets have sex-specific patterns of gene expression, with most differentially

expressed genes found on autosomes, demonstrating sex-specific regulation of shared genetic material²⁶. The sex chromosomes also directly contribute. Males are at higher risk of diseases caused by deleterious X-linked alleles. Incomplete inactivation of immunoregulatory genes on the X chromosome can also occur in females, which results in a gene dosage imbalance between sexes^{38,39}. Incomplete X chromosome inactivation has been implicated in female-biased autoimmune diseases⁴⁰ and in vaccine efficacy⁴¹. The Y chromosome has immunoregulatory function, broadly impacting immune transcriptional profiles linked to autoimmune disease⁴² and impacting outcomes of influenza virus and coxsackie virus infection in animals^{43,44}. Sex-specific features of epigenomic organization also dictate differential availability of

transcriptional targets^{21,45}. Superimposed on these genomic elements is the direct effect of sex steroid exposure. Oestrogens^{46,47}, progesterone⁴⁸⁻⁵² and testosterone⁵³ have direct effects on immune cell function that are driven by the signalling of these hormones through their respective cellular receptors. The variation in sex steroid concentrations that occurs over the life course contributes to differences in immune profiles and disease susceptibility patterns at different ages^{20,52}. Consistent with this variation, both sex and age contribute to unique transcriptional signatures of immune cells both at the baseline and after exposure to immunostimulants^{19,21,22}. The summative effect is a sex-specific transcriptional regulatory network of genetic variants, epigenetic modifications, transcription factors and sex steroids that leads to a functional difference

in the immune response^{35,51}. FIGURE 2 highlights intersections between SARS-CoV-2 infection and sources of sex bias in pathophysiology that warrant further investigation.

Sex bias in SARS-CoV-2 infection

Virus entry receptors. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as an entry receptor, with virus entry enhanced by cellular transmembrane serine protease 2 (TMPRSS2), which primes the spike protein of the virus^{55,56}. ACE2 is an X chromosome-encoded gene that is downregulated by oestrogens⁵⁷ and exhibits tissue-specific expression patterns⁵⁹. Differences in ACE2 expression may be driven by sex-differential expression of ACE2 variants^{58,60}. ACE2 is associated with interferon gene expression^{61,62}, which in turn shows sex-specific regulation. The cell-intrinsic regulation of ACE2 expression may change with age, in response to changing

levels of sex steroids, or following viral challenge. TMPRSS2 is regulated by androgen receptor signalling in prostate cells⁶³. Initial investigations have not demonstrated a significant difference in TMPRSS2 mRNA expression in lung tissue from males and females, but it is unknown whether androgens may impact expression in the setting of infection with SARS-CoV-2 (REFS^{63,64}) or whether the level of expression has an impact on SARS-CoV-2 burden. Further research is needed to determine whether sex-biased expression of ACE2, coupled with the regulation of TMPRSS2 by androgens, increases SARS-CoV-2 susceptibility of males compared with females.

Interferons. Innate sensing of viruses, production of interferons and activation of the inflammasome are the first line of defence against viruses⁶⁵. In the case of SARS-CoV-2, where there is no pre-existing adaptive immune memory, the success of

this early antiviral response may be a determinant of disease outcome. Innate sensing of viral RNA by the pattern-recognition receptor Toll-like receptor 7 (TLR7) is sex biased, as TLR7 escapes X chromosome inactivation, resulting in greater expression in female immune cells; this has also been linked to sex differences in autoimmunity^{66,67} and vaccine efficacy⁶⁸. There is greater production of interferon- α (IFN α) from plasmacytoid dendritic cells from adult females than from adult males^{67,68}, an effect modulated by sex steroids^{69–71}. In animal models of SARS-CoV infection, pretreatment with pegylated IFN α was associated with protection of lung tissue⁷² but without consideration of biological sex. In SARS-CoV-2, emerging data suggest that there is aberrant activation of interferon responses but preserved chemokine signalling, which has been postulated to contribute to immunopathology⁷³. Studies are needed

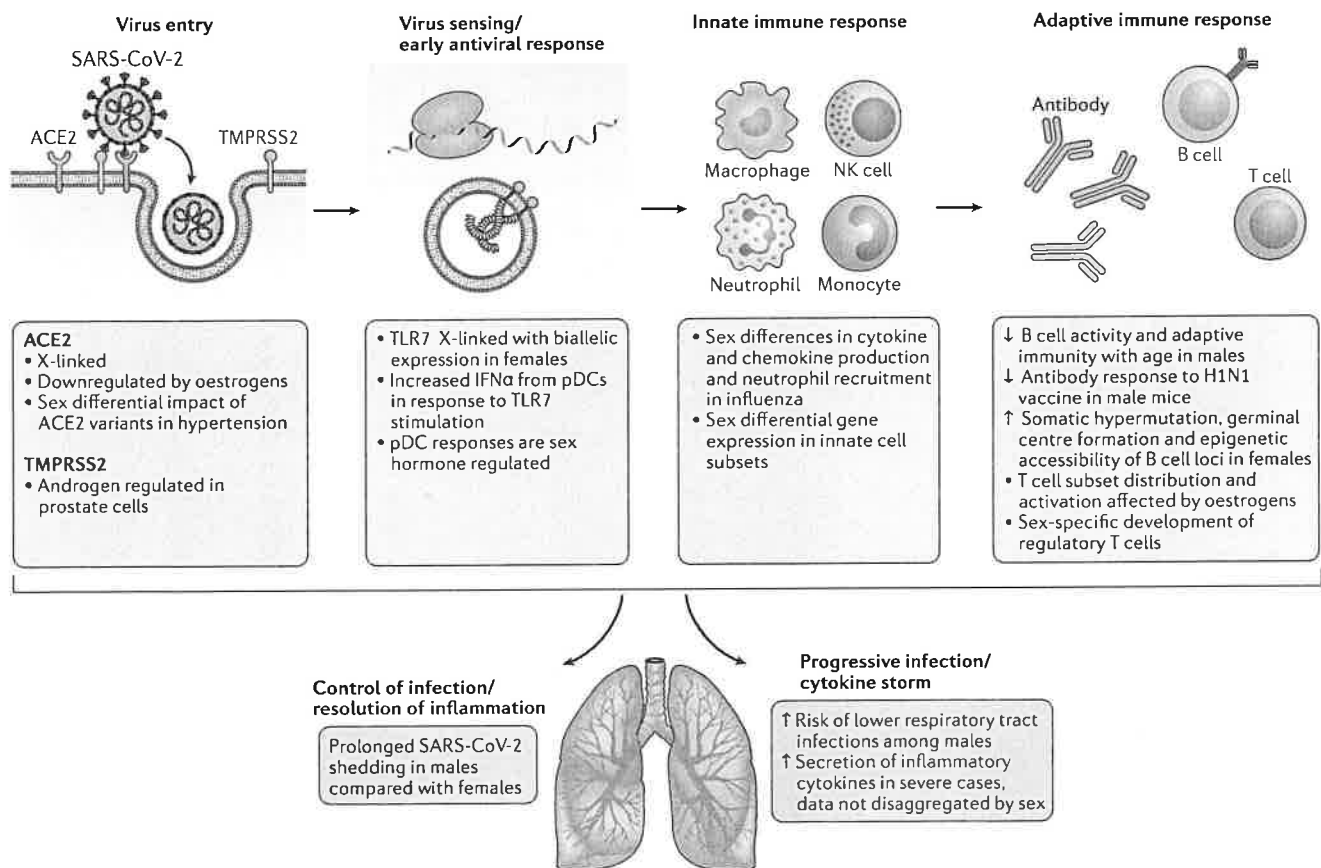


Fig. 2 | **Known sex differences that may impact immune responses to SARS-CoV-2 and COVID-19 progression.** An illustrative summary of the sequence of events in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the associated immune responses. Broadly speaking (from left to right), there are the initial steps of virus entry, innate recognition of the virus with activation of antiviral programmes, the recruitment of innate immune cells and induction of an adaptive immune response. These major steps culminate either in successful control of infection and pathogen elimination or in a pathological inflammatory state. Sex differences that may be operative at multiple points along these pathways are indicated in the blue boxes. ACE2, angiotensin-converting enzyme 2; H1N1, H1N1 influenza virus; IFN α , interferon- α ; NK, natural killer; pDC, plasmacytoid dendritic cell; TLR7, Toll-like receptor 7; TMPRSS2, transmembrane protease serine 2.

to determine whether differences in the magnitude or kinetics of the interferon response may contribute to a sex bias in the early control or severity of SARS-CoV-2 infection and may inform considerations of interferons as therapies for COVID-19 (REF.⁷⁴). Early data suggest that male sex may be associated with a longer duration of viral detection, even within families^{75,76}, raising the question of whether females have more efficient clearance of the virus. The rate of virus clearance will need to be assessed in evaluating the efficacy of innate and adaptive immune responses.

Adaptive immunity. Females generally mount greater antibody responses to viral infection and vaccination, albeit with higher levels of autoreactivity⁷⁷. The mechanisms for sex differences in antibody production include oestrogenic enhancement of somatic hypermutation⁷⁸, less stringent selection against autoreactive B cells^{77,79–82} and sex differences in germinal centre formation⁸³ and in the epigenetic accessibility of B cell loci²¹. It is still unknown whether sex has an impact on antibody generation in SARS-CoV-2 infection. Early studies suggest that titres of antibodies to some viral epitopes are higher in patients with severe COVID-19 and that seroconversion may not be tightly linked to declining virus titres^{84,85}. Ongoing studies evaluating the infusion of convalescent serum may provide answers as to the protective capacity of these antibodies⁸⁶, but these studies are currently not considering biological sex. Generation of protective, neutralizing antibodies is a goal of vaccine development, with the cautionary note that in models of SARS-CoV vaccination some antibody responses induced potent inflammatory responses⁵⁷. Persistence of antibodies, epitope targeting and non-neutralizing Fc-mediated antibody characteristics should be assessed with sex-stratified analyses. As vaccines are developed, the female bias towards both potent responses and adverse effects should be considered and sex-specific dosing should be tested, where appropriate⁸⁷.

Sex impacts the development of regulatory T cells^{88–91}, the distribution of lymphocyte subsets⁹² and the overall quality of T cell responses^{93,94}. In T cells, overexpression of X-encoded immune genes, including *CD40LG* and *CXCR3*, has been linked to incomplete X chromosome inactivation and T cell-specific epigenetic modifications of the X chromosome^{95,96}. It is unknown whether T cell phenotypes contribute to COVID-19; data from the prior SARS outbreak did not link T cell

responses to outcomes in humans⁹⁷, but mouse models suggest a role for CD4⁺ T cells⁹⁸. In patients with MERS, T cell responses were dysregulated⁹⁹, but sex differences were not analysed. In the current pandemic, lymphopenia is associated with severe disease^{100,101}, and early evidence suggests that the clinical markers of lymphocyte count may be lower in males and neutrophil–lymphocyte ratios may be higher¹⁷. Further work is needed to define the sex-differential role of T cells in acute infection, in acute lung injury phenotypes¹⁰² and as potential vaccine targets.

Severe infection and acute respiratory distress syndrome. Severe cases of COVID-19 are typically marked by acute respiratory distress syndrome (ARDS), with respiratory failure requiring oxygen support and mechanical ventilation. The infection is primarily characterized by diffuse alveolar damage without a consistent pattern of cell infiltration^{75,103–105}. The pathogenesis of ARDS involves the disruption of normal barrier function, inflammation and subsequent tissue repair. Whether there are sex-specific risks for ARDS and death from other causes, such as trauma, remains unknown^{106,107}, although there is a suggestion of a higher risk of lower respiratory tract infections among males¹⁰⁸ and that steroid hormones modulate the immune response to respiratory viral pathogens¹⁰⁹. In one cohort of patients with COVID-19, severe respiratory failure was associated with a pattern of inflammation, macrophage activation and depletion of lymphocytes that was distinct from bacterial infection¹¹⁰. There was a sex bias for severe COVID-19 not observed in the comparator group with bacterial infections¹¹⁰. Sex-differential production of IL-6 (REF.¹¹¹), monocyte transcriptional patterns and inflammatory set point^{19,21,22} could contribute to an enhanced risk of death in males and highlight the importance of sex-stratified analyses to guide deployment of safe and effective immunomodulatory therapeutics for males and females¹¹².

Conclusions

Emerging data demonstrating more favourable outcomes for community-dwelling adult females across age strata offer an immediate opportunity for comparative biology experiments to define features of COVID-19 pathogenesis and the associated immune response. The research pipeline should integrate sex as a biological variable in all stages, from fundamental research to preclinical

drug development, clinical trials and epidemiological analyses¹¹³. Considering the role of intersecting factors — including, but not limited to, gender, age, race and other identifying characteristics — is critical to understanding the biological and sociocultural factors contributing to heterogeneous COVID-19 outcomes. Sex is a driver of discovery and innovation¹¹⁴, and taking a sex-informed approach to COVID-19 research¹¹⁵ and medicine¹¹⁶ will uncover novel features of the host immune response to SARS-CoV-2 and ultimately result in more equitable health outcomes.

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<https://doi.org/10.1038/s41577-020-0548-8>

Published online: 11 June 2020

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Immunology thanks E. Fish, P. McCombe and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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